UDC 631.417.2: 631.95

DOI: 10.15372/CSD2019187

Evaluation of the Biological Activity of Humic Preparations under the Conditions of Technogenic Landscapes

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Abstract

The biological activity of humic acids produced from the coals of the Kansk-Achinsk and South Ural basins was evaluated by means of phyto-testing in a series of laboratory and field tests. A connection between the structural group parameters of the organic acids of humic preparations (humic acids) and their biological activity was revealed in the series of field experiments at the sites characteristic of the most widespread technogenic landscapes of Siberia. It was established that in the case when humates are applied at the burrows of open-pit coal mines with the lack of moisture, it is necessary to take into account the hydrophobic-hydrophilic parameters of the preparations. Preparations with the high degree of aromaticity of humic acids are more efficient for the reclamation of the burrows of brown coal deposits.

Keywords: humic preparations, biological activity, technogenic landscape, reclamation, phytoactivity index

INTRODUCTION

It is known that the growth of the production of mineral resources inevitably leads to an increase in the rate of transformation of natural landscapes into technogenic ones and, as a consequence, to the ecological imbalance in coal-producing regions [1]. At the same time, coal and wastes from the coal industry may be used to obtain humic acids (HA) possessing high biological activity. In this respect, especially important are wastes, which are naturally oxidized (nonstandard) forms of run-of-mine grades of coal [2]. It was established that the efficiency of the isolation of humic preparations (HP), which are the salts of HA (humates), from these kinds of coal is higher than that of similar preparations but obtained from conditioned coal [3]. The high biological activity of HP allows using them as the stimulators of plant growth and development during land reclamation [4–6].

The goal of the studies was to reveal a connection of the biological activity and structural group composition of HP and evaluate its manifestation under the conditions of technogenic landscapes.

The paper is a continuation of the series of works aimed at the studies of the biological activity of humic substances obtained from brown coal [7].

EXPERIMENTAL

Evaluation of the biological activity of HP was carried out using wheat seeds in two stages: in the laboratory experiment and under the conditions of technogenic landscapes represented by the dumps of the open-pit mine Zarechniy of JSC

SUEK-Kuzbass. During the investigation, not only effects stimulating plant development were determined for different versions but also suppression of one or another test function. Since the use of concentrated HP solutions has a suppressive action on plants, the solutions were diluted to 0.02 %. Under laboratory conditions, the biological activity of HP was evaluated from three test functions: the energy of seed germination, root length, and seedling length. To formalize the obtained values of test functions, the integral index of phytoactivity (IP) of HP was calculated. This index depicts the deviations of the value of test function from the reference (distilled water) [8]. The index of phytoactivity is a generalizing parameter and is calculated as an average of the sum of the parameters of seed germination energy, root length and seedling length, expressed in the fractions of a unit.

Under the conditions of field experiment, wheat seeds were wetted in HP solution for one day, and then the seeds were sown. The plots for field experiments were chosen relying on the properties corresponding to rock dumps of the most widespread technogenic landscapes of Siberia. Because of this, when laying experimental grounds, we chose the substrates represented by the technogenic eluvium of dense sedimentary rocks comprising the major part of coal deposits. The substrates of the second version were loess-like loamy soils (loose sedimentary rocks similar to those forming the dumps of brown coal open-pit mines). The use of these substrates containing insignificant amount of the carbon of organic substances (less than 3 %) allows a more reliable evaluation of the effect from the action of HP. For comparison, the substrates of organogenic rocks enriched with humic substances of natural origin (peat) were chosen as the third version. The versions of the chosen substrates differ in physical and chemical properties (Table 1) due to the characteristics of the rocks comprising them. These properties affect the moisture content in the experimental plots. Experiments were laid in triple on plots with the area of 2 m2. For reference versions, distilled water was used instead of humate solutions in the same amounts as in the versions with the preparations.

TABLE 1

Major physical and chemical properties of the substrates

Properties	Substrate		
	Dense rocks	Loose rocks	Organogenic rocks
Stoniness, %	61	0	0
Density, g/cm^3	1.6	1.2	0.83
Content of organic carbon, %	2.7	3.1	30.3
pH_{aq}	7.5	7.2	6.8

Humic preparations were obtained from brown coal of conditional grades and its naturally oxidized forms. In particular, we chose humus-containing brown coal from the Tisul deposit of the Kansk-Achinsk basin (BUTS), its naturally oxidized form (BUTSO), coal from the Tyulgan deposit if the South Ural basin (BUT). The humates of sodium (HumNa) or potassium (HumK) from different samples of brown coal were obtained by precipitation from the alkaline solution after the addition of hydrochloric acid [9]. Humate samples were characterized by means of functional analysis and ^{13}C NMR spectroscopy (Table 2). High-resolution ^{13}C NMR spectra in solid were recorded with the help of the AVANCE III 300 WB spectrometer (Bruker, Germany) at the frequency of 75 MHz using the standard procedure of cross polarisation and magic angle spinning (CPMAS).

On the basis of the analysis of literature data [10–12], to reveal a connection of the biological

TABLE 2

Integral intensities of spectral regions and structural parameters of humic preparations according to ^{13}C NMR data [7], %

Sample	Chemical shift, ppm							Structural parameter		
	220–187	187–165	165–145	145–108	108–90	90–48	48–5	f_a	$f_{h/h}$	$f_{ar/al}$
	C=O	COOH(R)	C_{Ar-OH}	C_{Ar}	$C_{O-Alk-O}$	C_{Alk-O}	C_{Alk}			
HumNa BUT 30	1.5	6.4	8.5	26.0	5.2	15.2	37.3	34.5	0.6	0.6
HumNa BUT 31	2.4	7.6	8.5	25.2	4.2	12.2	39.9	33.7	0.5	0.6
HumNa BUTSO	3.5	7.4	8.2	31.7	6.3	14.8	26.8	39.9	0.7	0.8
HumK BUTSO	3.8	6.7	9.5	33.1	6.3	14.0	25.4	42.6	0.7	0.9
HumNa BUTS	1.0	8.2	6.7	51.1	3.4	7.3	22.4	57.8	0.4	1.7

activity of HP with the structural group composition, we chose three parameters calculated from the data of ^{13}C NMR spectra (CPMAS):

1) degree of aromaticity

$f_a = C_{Ar-OH} + C_{Ar}$

2) hydrophilic-hydrophobic parameter

$f_{h/h} = (C{=}O + COOH(R) + C_{Ar-OH} + C_{O-Alk-O} + + C_{Alk-O}) / (C_{Ar} + C_{alk})$

3) aromaticity/aliphaticity

$f_{ar/al} = (C_{Ar-OH} + C_{Ar})/(C_{O-Alk-O} + C_{Alk-O} + C_{alk})$

Statistical processing of experimental data was carried out by means of determination of Spearman correlation coefficient with the help of Microsoft Office Excel 2007 software.

RESULTS AND DISCUSSION

Results of laboratory experiments aimed at the evaluation of HP effect on the development of wheat show that the application of the majority of the compounds under investigation has a positive effect on the energy of seed germination, root length and seedling length (Fig. 1). A negative effect on the length of wheat roots was caused by HP obtained from the brown coal of the Tyulgan deposit (HumNa BUT 31). Wheat seed wetting in humate solutions had the strongest effect on seedling length. As a result, the IP of HP was 1.30 for HumNa BUT 30, 0.94 for HumNa BUT 31, 1.20 for HumNa BUTSO, 1.21 for HumK BUTSO, 1.38 for HumNa BUTS. The maximal values are characteristic of sodium humates obtained from brown coal of the Tisul (HumNa BUTS) and Tyulgan (HumNa BUT 30) deposits.

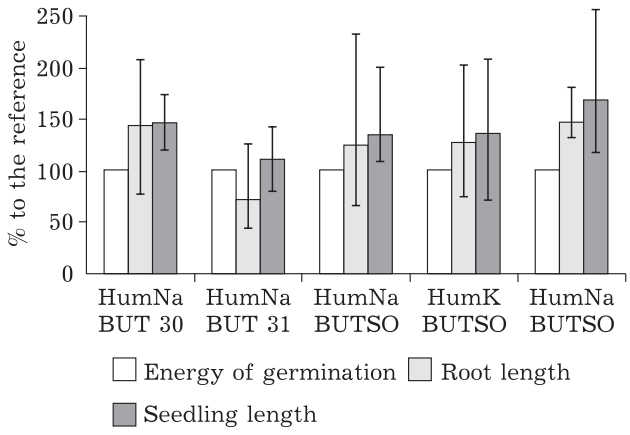

Fig. 1. Values of test functions of wheat young growth.

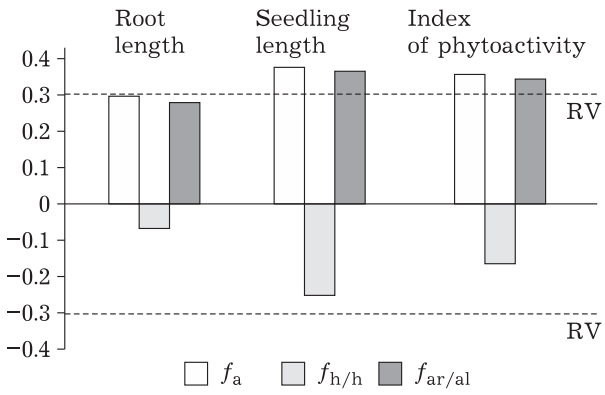

Fig. 2. Spearman correlation coefficient (n = 40, where n is the number of ranked parameters) between the parameters of test functions of wheat and the structural parameters of the samples of humic preparations. RV is the boundary of the region of reliable values.

The statistical treatment of the data obtained in laboratory experiments showed a reliable correlation of seedling length (Fig. 2) with parameters f_a and $f_{ar/al}$, which are defined by the integral intensities of spectral regions depicting the fractions of carbon C_{Ar}, C_{Ar-OH} and C_{Alk} (Fig. 3). However, the correlation coefficients between the amount of C_{Ar-OH} and C_{Alk} and the values of test functions for wheat seedlings are characterized by minimal values of significance limits (see Fig. 2, 3).

Evaluating a correlation between HP properties and IP, it is necessary to stress that the correlation dependence is observed only with the parameter determined from the chemical shift in the NMR spectrum within the range 5–48 (C_{Alk}) and 165–187 (C_{Ar}) ppm (see Fig. 3). The correlation coefficient of the linear function describing the dependence is equal to 0.36 and 0.37, respectively.

Results of field tests carried out with three kinds of substrates allowed us to evaluate the efficiency of HP for wheat seedlings (Table 3). It was established that the majority of HA cause a positive influence on plants on dense and loose rocks with insufficient and optimal wetting during experiments, respectively. The number of wheat young-growth plants on the plot with organogenic rocks was smaller in comparison with the reference, which is likely to be affected by the presence of the humic substances of peat in the soil solution. Sodium humates from coal of the Tisul deposit (HumNa BUTSO and HumNa BUTS) had a positive effect on the young growth to a higher extent. A negative effect on the germinating capacity of wheat was detected in the case of the use of potassium humate (HumK

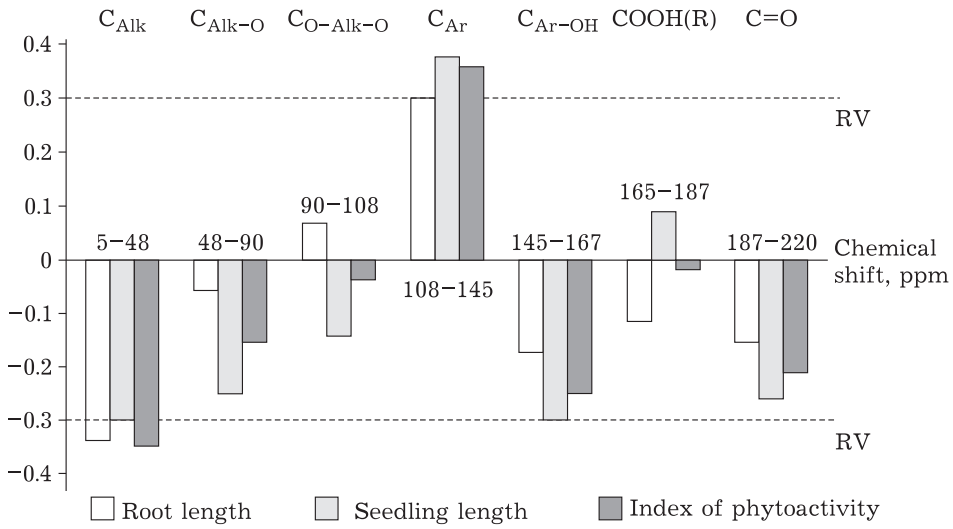

Fig. 3. Spearman correlation coefficient (n = 40, where n is the number of ranked parameters) between the parameters of test functions of wheat and the intensity of the spectral regions of humic preparations. RV is the boundary of the region of reliable values.

BUTSO) and HP isolated from coal of the Tyulgan deposit (HumNa BUT 30).

The mentioned trend of the multidirectional effect of HP manifested itself also in the phase of the third leaf and in the phase of tillering. Systematization of the results and their correlation with humate properties revealed some dependences (Table 4). The hydrophilic-hydrophobic parameter $f_{h/h}$ of HA demonstrated a negative correlation with the height of wheat stalks on plots with dense sedimentary rocks of coal deposits. Under the conditions of less pronounced deficit of moisture on loose sedimentary rocks, reliable positive values for the correlation of this parameter were recorded only for the phase of sprouts. A minimal correlation $f_{h/h}$ with the phase indices of wheat was obtained for the substrates of organogenic rocks.

TABLE 3

Effect of the salts of humic acids from brown coal on the amount of wheat young growth on the substrates of technogenic landscapes (% with respect to the reference)

Version	Substrate		
	Dense rocks	Loose rocks	Organogenic rocks
HumNa BUTS	134.2	168.2	96.5
HumNa BUTSO	489.5	236.4	90.7
HumNa BUT 30	76.3	81.8	65.3
HumNa БУТ 31	118.4	136.4	78.4
HumK BUTSO	78.9	70.5	65.6

The degree of aromaticity f_a, as well as parameter $f_{ar/al}$ characterising the ratio of aromatic to aliphatic components of humic acids, demonstrated a reliable correlation with the height of wheat young growth at the phase of the third leaf under the conditions similar to those for the dumps of brown coal open-pit mines composed of loose sedimentary rocks, and in all versions with organogenic rocks under the conditions of excess

TABLE 4

Spearman correlation coefficient (n = 15, where n is the number of ranked parameters) between the parameters over the phases of wheat development and structural parameters of the samples of humic acid salts

Development phase	Structural parameters		
	$f_{h/h}$	f_a	$f_{ar/al}$
	Dense rocks		
Young growth	−0.50	0.18	0.23
3rd leaf	**−0.72**	0.04	0.11
Tillering	−0.09	0.25	0.27
	Loose rocks		
Young growth	**0.58**	−0.33	−0.35
3rd leaf	−0.22	**0.76**	**0.74**
Tillering	0.37	−0.02	−0.04
	Organogenic rocks		
Young growth	0.49	**−0.97**	**−0.95**
3rd leaf	−0.02	**0.65**	**0.62**
Tillering	−0.52	**0.64**	**0.66**

Note. Reliable values are printed in bold.

wetting (see Table 4). In the latter case, the detected correlation changes its sign from the negative to positive, which may be a result of the prolonged action of HP under test.

In general, the data obtained in field tests allow us to state that the presowing treatment of seeds with the preparations under investigation affects the growth and development of wheat. Under the conditions of technogenic landscapes, in the case of sufficient and excessive wetting, biological activity is determined by the degree of aromaticity f_a of HA. In technogenic landscapes with a wetting deficit, a connection between the phase parameters of wheat young growth and the hydrophilic-hydrophobic parameter $f_{h/h}$ is most clearly pronounced.

CONCLUSION

Investigation of the manifestation of biological activity of humic preparations under the conditions of technogenic landscapes showed that the majority of substances under test are able to cause a positive effect on the growth and development of wheat seeds. The preparations demonstrated the most clearly pronounced biological activity under the conditions of the laboratory experiment: the maximal efficiency was manifested by sodium humates isolated from brown coal of the Tisul deposit in the Kansk-Achinsk basin (HumNa BUTS) and from coal of the Tyulgan deposit in the South Ural basin (HumNa BUT 30). Laboratory experiments revealed the effect of the preparations on an increase in the length of wheat roots and seedlings. Statistical treatment of the data obtained in the experiments demonstrated a reliable correlation of seedling length with the degree of aromaticity f_a and the parameter characterising the ratio of aromatic to aliphatic groups $f_{ar/al}$, which was determined for the studied samples of brown coal.

Under the conditions of the field experiment, the highest biological activity was demonstrated by sodium humates isolated from the naturally oxidized form of brown coal from the Tisul deposit of the Kansk-Achinsk basin (HumNa BUTSO), to a smaller extent – their run-of-mine analogues (HumNa BUTS), as well as humic preparations obtained from coal of the Tyulgan deposit in the South Ural basin (HumNa BUT 31). The mentioned preparations exhibited a reliable positive effect under the conditions of insufficient and optimal wetting – on dense and loose sedimentary rocks, respectively. Under the conditions of excess wetting that existed on the plots with organogenic rocks, the efficiency of humates under test was negative or close to reference at the stage of young growth.

The revealed dependences of the parameters of structural group composition of humic preparations and their biological activity manifested during different stages of wheat development allow us to conclude that the choice of raw material and methods of obtaining preparations used for reclamation should be aimed at the formation of properties corresponding to edaphic and climatic conditions of the territory of their application. In the case if humates are used on the dumps of coal open-pit mines with the deficit of moisture, it is necessary to take into account the properties of preparations determining their hydrophilic-hydrophobic parameters. Under the conditions of the dumps of brown coal deposits, which are usually characterized by optimal and excess wetting of the substrates, preparations with a higher degree of humic acid aromaticity are more efficient.

Acknowledgements

The work was carried out within the framework of the basic project in the ISSA SB RAS and under the financial support from the RFBR (Project No. 18-55-91033).

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